EXHIBIT K

Dietary *N*-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study[1–3]

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ABSTRACT

Background: Dietary *N*-nitroso compounds and endogenous nitrosation are important carcinogenic factors, but human evidence of their role is scarce for esophageal cancer and inconsistent for gastric cancer.

Objective: We studied the relation between risks of esophageal and gastric cancer subtypes and dietary intake of *N*-nitrosodimethylamine, heme iron, nitrite, and nitrate in the Netherlands Cohort Study.

Design: A total of 120,852 men and women aged 55–69 y were recruited in 1986, and diet, based on a 150-item food-frequency questionnaire, and other risk factors were assessed. The cohort was followed for 16.3 y, and 110 esophageal squamous cell carcinoma (ESCC), 151 esophageal adenocarcinoma, 166 gastric cardia adenocarcinoma, and 497 gastric noncardia adenocarcinoma (GNCA) cases were analyzed along with 4032 subcohort members in a case-cohort analysis.

Results: Positive associations were observed between *N*-nitrosodimethylamine intake and ESCC risk (HR for 0.1-μg/d increase in intake: 1.15; 95% CI: 1.05, 1.25; *P*-trend = 0.01 based on tertiles of intake) and GNCA risk (1.06; 95% CI: 1.01, 1.10; *P*-trend = 0.09) in men. ESCC risk was associated with nitrite intake (HR for 0.1-mg/d increase: 1.19; 95% CI: 1.05, 1.36; *P*-trend = 0.06) and heme-iron intake (HR for 1-mg/d increase: 1.83; 95% CI: 0.98, 3.39; *P*-trend = 0.03). Among women, exposure levels were lower, and we found no convincing positive associations.

Conclusion: These results suggest that *N*-nitroso compounds may influence the risk of ESCC in men, but there are no clear associations for other esophageal and gastric subtypes.   *Am J Clin Nutr* 2013;97:135–46.

INTRODUCTION

N-Nitroso compounds (NOCs)[4] are a group of naturally occurring compounds containing a nitroso group bound to a nitrogen atom (1). Humans are exposed to NOCs mainly through diet, cigarette smoking, occupational exposure, and endogenous nitrosation. Several NOCs are carcinogenic in animals (2), and the most frequently occurring carcinogenic NOC in foods—*N*-nitrosodimethylamine—is classified as probably carcinogenic to humans (group 2A) by the International Agency for Research on Cancer (3). The major dietary sources of *N*-nitrosodimethylamine are cured meat, pickled fish and vegetables, and food products dried by using direct-fire methods. Beer used to be an important source of *N*-nitrosodimethylamine. Endogenous formation of NOC by nitrosation of secondary amines via nitrite under acidic

conditions is another major source of exposure, and it is estimated to contribute 45–75% of the total NOC exposure (1). Endogenous NOC formation in humans in the lower gastrointestinal tract is also influenced by the heme-iron content of consumed meat (4), and it has been shown in rats that chlorophyll inhibits the effect of heme in the gastrointestinal tract (5).

Although there is convincing evidence from animal studies for the role of exogenous NOCs in the etiology of several cancers, including esophageal and gastric cancer (2), and most of the case-control studies suggest a positive association between nitrosamines, nitrite intake, and the risk of gastric cancer (6, 7), cohort studies are much less conclusive (7–9) and few studies have investigated associations with the risk of esophageal cancer (7, 10). Inconsistencies might be partly due to the different etiologies of esophageal and gastric cancer subtypes and the possible effect modification by inhibitors of endogenous nitrosation, such as vitamin C. We also hypothesized that chlorophyll, which is structurally similar to heme, may influence the effect of heme on endogenous NOC formation.

In this study, we assessed the association between the risk of esophageal and gastric cancer subtypes and exposure to *N*-nitrosodimethylamine, heme iron, nitrite, and nitrate intakes in a large Dutch cohort recruited in 1986, which was likely to have experienced a wide range of *N*-nitrosodimethylamine exposure because beer in the Netherlands still contained a considerable amount of *N*-nitrosodimethylamine before the 1980s (11, 12). We also examined the effect modification by vitamin C and chlorophyll intake.

SUBJECTS AND METHODS

Design and study population

The Netherlands Cohort Study (NLCS) is a prospective cohort study started in September 1986. At baseline 58,279 men and 62,573 women aged 55–69 y were recruited (13). The sample was selected from 204 municipal population registries throughout the Netherlands by sex-stratified random sampling. At baseline, a self-administered questionnaire was completed by study participants on dietary habits and other risk factors of cancer. A case-cohort design is used for processing and analyzing data (14); hence, a random subcohort was selected immediately after identification of cohort members. The choice for the size of the subcohort ($n = 5000$) was based on relative efficiency comparisons of RRs that would be obtained from a full cohort study compared with a case-cohort design (13). The subcohort is followed for migration and vital status to estimate the accumulated person-years of the whole cohort. Only one subcohort member was lost during 16.3 y of follow-up. Ethical approval to conduct the study was obtained from the institutional review boards of the University Hospital Maastricht and TNO Nutrition and Food Research.

Follow-up of cases

Incident cases of cancer in the entire cohort throughout 16.3 y of follow-up were identified by using annual computerized record linkage to the Netherlands Cancer Registry and the Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (15). The completeness of the cancer registries was estimated to be >95% (16). A total of 336 cohort members emigrated from the Netherlands during the study. Endpoints for this study were incident, microscopically confirmed esophageal and gastric cancer cases classified by anatomic site and histologic type defined by the International Classification of Diseases for Oncology, Third Edition (17). Esophageal carcinomas included squamous cell carcinomas C15 (histology codes 8050–8076) and adenocarcinomas C15 (histology codes 8140, 8141, 8190–8231, 8260–8263, 8310, 8430, 8480–8490, 8560, and 8570–8572). Gastric cancer was classified as cardia adenocarcinomas C16.0 and non-cardia adenocarcinomas as C16.1–C16.9, including overlapping (C16.8) and not otherwise specified (C16.9) tumors. The distribution of cancer subtypes in the cohort and the selection of cases and subcohort members are shown in **Figure 1**. Individuals with prevalent cancer (except skin cancer) at baseline and those with incomplete and/or inconsistent dietary data (18) were excluded from the analysis.

Assessment of determinants

The baseline questionnaire included 150 items on food and beverage consumption during the year before the start of the study. Data were key-entered and processed in a standardized manner. To minimize observer bias, data entry was blinded with respect to case/subcohort status. The questionnaire was validated against a 9-d diet record (18). Spearman correlation coefficients for alcoholic beverages, meat products, and vegetables, which are major sources of NOCs and nitrate in the diet, were 0.89, 0.54, and 0.38, respectively.

N-nitrosodimethylamine

N-nitrosodimethylamine values in food items and food groups were initially extracted from publications in which the
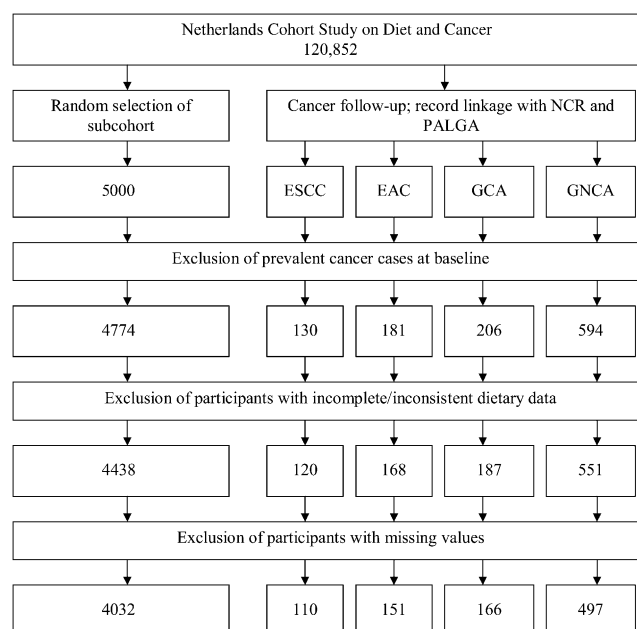
FIGURE 1. Flowchart of study participant selection and follow-up in the Netherlands Cohort Study on Diet and Cancer, 1986–2002. EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric noncardia adenocarcinoma; NCR, Netherlands Cancer Registry; PALGA, Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands.

N-nitrosodimethylamine content was measured in Dutch foods in the 1970s and 1980s. Publications were searched by using PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and Web of Science (webofknowledge.com), 2 previously developed food databases of nitrosamines (19, 20), and references from identified publications. The following information was extracted when available: type and number of food samples analyzed, number of samples that were positive for N-nitrosodimethylamine, detection method, source of sample, preparation of sample, and mean and range of N-nitrosodimethylamine content. Food items for which N-nitrosodimethylamine values were not available from Dutch sources, but nonzero content was indicated in a comprehensive food-composition database of nitrosamines (20), measurements made in food sources from Western or northern Europe in the 1980s were used (21, 22). We excluded studies in which food samples for N-nitrosodimethylamine measurements were selected according to some specific characteristic of the source of the food item (eg, animals fed a high-nitrate diet).

The collected N-nitrosodimethylamine data for food items were used to assign a final N-nitrosodimethylamine value for food items used in the NLCS cohort. If values for a food item were available from several sources or from different types (eg, types of cheese), a weighted average value was calculated by using the number of analyzed samples as a weight. For food items containing more than one ingredient, N-nitrosodimethylamine values were calculated by using the proportion of N-nitrosodimethylamine–containing ingredients in the recipe of the food item. When N-nitrosodimethylamine values were not available for a food item, the value for the closest comparable food were assigned (eg, alcohol free beer—beer). N-nitrosodimethylamine values in food items together with the frequency of consumption and serving

sizes were used to calculate N-nitrosodimethylamine intake for subcohort members and cases.

Nitrate and nitrite

Food-composition values for nitrate were derived from the databank on contaminants in food from the State Institute for Quality Control of Agricultural Products (RIKILT; Wageningen, Netherlands) (23). Estimations were based on the mean nitrate contents between 1985 and 1989. Distinction between summer and winter was made for some vegetables [eg, endive (raw and cooked), lettuce], and information on nitrate losses during preparation (washing, cutting, and cooking) were considered. For several vegetables, experimental data were available regarding nitrate losses during preparation (24, 25). The percentage nitrate losses used to construct the nitrate table were 16%, 31%, 42%, 20%, and 49% for endive, spinach, chicory, cabbage, and potatoes, respectively. For other vegetables consumed after cooking, nitrate losses were estimated to be 40%. For lettuce, a 20% loss was used. Information about nitrate content in drinking water from all pumping stations in the Netherlands in 1986 (Vereniging van Exploitanten van Waterleiding bedrijven in Nederland 1989) was used to determine the nitrate concentration in drinking water for each home address by postal code. To calculate the nitrate intake from water, we used information from the questionnaire about the amount of water, coffee, tea, and soup consumed. Total nitrate intake was calculated by summing dietary intake and nitrate from water. The median proportion of dietary nitrate to total nitrate was 99% in the subcohort, and the major source of dietary nitrate intake was from leafy vegetables.

Food-composition values for nitrite were obtained from analyses conducted by the National Public Health Institute in 1984, based on 5 samples per product (26). Nitrite intake was assessed solely on the intake of processed meat, because the nitrite content of vegetables and cheese was considered negligible in comparison with processed meat.

Heme iron

Daily heme iron intake was derived from the heme-iron content of food items calculated as a product of total iron content and mean percentage heme iron relative to total iron in each specific origin of meat. Total iron content was obtained from the Dutch food-composition table (27), and type-specific percentages of heme iron were estimated from studies measuring total and heme iron in the same meat samples (28). The average percentages of heme iron relative to total iron were 65%, 39%, and 26% for cooked beef, pork, and chicken or fish, respectively.

Chlorophyll

Intake of chlorophyll was assessed by using vegetable intake and previous reports on chlorophyll content in vegetables described in detail by Balder et al (28).

Vitamin C

Daily vitamin C intake was calculated from the food-frequency questionnaire data by using the Dutch food-composition table (27).

Index of endogenous NOC formation

An index of endogenous NOC (ENOC) was previously determined by Jakszyn et al (8) based on a strong correlation ($r =$ 0.95) between intake of iron from meat and measured apparent total NOC formation (ATNC) in fecal samples of individuals administered a controlled diet. We estimated ENOC by calculating the heme-iron content of diets used in controlled experiments that measured ATNC in fecal samples (4, 29–32) and correlated heme-iron content with corresponding ATNC measurements ($r = 0.97$). On the basis of the strong correlation, we used linear regression to predict the ENOC of cases and subcohort members using their heme iron intake [ENOC (μg/d) = 45.05 + 49.23 $\times$ heme iron] for observations of heme iron $\leq$4.05 mg/d. For 3 subcohort members and 1 member with squamous cell carcinoma who had a heme iron intake >4.05 mg/d, we used a value of 244.4 μg/d (predicted by the model) because extreme high values of heme iron did not yield good predictions. We present ENOC estimates for categories of heme iron intake.

Assessment of potential confounders

Information on education (primary school, lower vocational, high school, or higher vocational/university); cigarette smoking status (never, ex-, or current smoker); smoking history (number of cigarettes smoked and duration of smoking); total energy intake (kJ/d); BMI (in kg/m^2); nonoccupational physical activity (<30 min/d, 30–60 min/d, 60–90 min/d, or >90 min/d); beer, alcohol, vegetable, and fruit consumption; long-term (>0.5 y) use of nonsteroidal antiinflammatory drugs and lower esophageal sphincter–relaxing medications (nitroglycerins, aminophyllines, β-blockers, anticholinergics, nifedipine, and benzodiazepines); and history of esophageal or gastric cancer in the family were derived from the baseline questionnaire.

Statistical analysis

Means, SDs, medians, IQRs, and frequencies were used to describe data in cases and subcohort members. Categories of intakes were constructed by using tertiles based on the respective distributions in the subcohort. The Cox proportional hazards model was used to estimate HRs and 95% CIs for esophageal and gastric cancer subtypes by comparing categories of intakes with the use of listwise deletion of cases with missing data; continuous intake variables (increment of 0.1 μg/d, 1 mg/d, 0.1 mg/d, 10 mg/d for N-nitrosodimethylamine, heme iron, nitrite, and nitrate, respectively) were also used. SEs were calculated by using a robust variance estimator (33). The proportional hazard assumption was assessed by using the scaled Schoenfeld residuals (34) and by plotting –ln [–ln (estimated survivor function)] as a function of time on the logarithmic scale (35). Stratified Cox models were used when combining men and women to allow different baseline hazards. All models were adjusted for age, and additional models were adjusted for smoking status (current yes or no), number of cigarettes smoked per day, duration of smoking (y), total energy intake (kJ/d), BMI (in kg/m^2; <20, 20–24.9, 25–29.9, or $\geq$30), vegetable intake (g/d), fruit intake (g/d), levels of education (4 categories), and nonoccupational physical activity (4 categories) and further for alcoholic beverages not including beer (g/d) for models of N-nitrosodimethylamine intake and alcohol (g/d) for all other models. Moreover, models for esophageal adenocarcinoma were also adjusted for long-term use of lower esophageal sphincter–relaxing medications (yes or no). Tests of linear trend in the HRs were performed by fitting models with the median values of each exposure category as a continuous variable.

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We tested for effect modification of vitamin C and chlorophyll intake by adding cross-product terms in the regression models. The Wald statistic was used to test for an interaction. Because of the low number of cases among women, these analyses were not performed for esophageal and gastric cardia adenocarcinoma in women.

We have carried out several sensitivity analyses to assess the robustness of our findings. First, the analysis was conducted by excluding the first 2 y of follow-up to assess possible effects of preclinical disease on the associations. Second, we used age as time scale in the Cox models to assess possible bias due to the choice of time scale. Third, we conducted analyses restricted to individuals who were not drinking beer. Fourth, because many dairy products were eaten in the NLCS and we had few estimates of *N*-nitrosodimethylamine content in dairy products other than cheese, we performed the analysis with *N*-nitrosodimethylamine calculated by using only food products other than noncheese dairy products. All analyses were performed by using the Stata statistical software package [Stata/IC for Windows (32-bit), version 12.0; StataCorp].

RESULTS

In total, 924 cases and 4032 subcohort members were analyzed with an average follow-up of 14.3 y in the subcohort. The baseline characteristics of the case groups and subcohort members are shown in **Table 1**. Those with esophageal squamous cell carcinoma were more likely to be current smokers and consumed more alcohol than subcohort members. *N*-nitrosodimethylamine intakes ranged from 2 to 2701 ng/d (mean: 109 ng/d, median: 58 ng/d) in the subcohort, with substantially higher intakes in men than in women. *N*-nitrosodimethylamine, heme iron, and nitrite intakes and ENOC levels were higher and nitrate intakes were lower among men with esophageal squamous cell carcinoma than in men in the subcohort.

A total of 73% of the *N*-nitrosodimethylamine intake in the subcohort was from beer and processed meat, with a marked contribution of beer in individuals with a high *N*-nitrosodimethylamine intake among men (**Figure 2**). Other sources of *N*-nitrosodimethylamine included dairy products, meat, coffee, and fish.

In men, the *N*-nitrosodimethylamine intake was positively associated with the risk of esophageal squamous cell carcinoma in age-adjusted and multivariable-adjusted models (HR for the highest tertile in the multivariable model: 2.43; 95% CI: 1.13, 5.23; *P*-trend = 0.01), and there was a weaker association with gastric noncardia adenocarcinoma, but not with esophageal adenocarcinoma or gastric cardia adenocarcinoma (**Table 2**). The multivariable-adjusted model with continuous exposure variable estimates a statistically significant 15% increase in risk of squamous cell carcinoma of the esophagus per 0.1-μg increase in daily *N*-nitrosodimethylamine intake. Among women, models with continuous *N*-nitrosodimethylamine exposure showed a weak significant association with esophageal squamous cell carcinoma (Table 2). However, tests for trend were not significant. No association was seen for esophageal adenocarcinoma or gastric cancer subtypes. A combined analysis of men and women showed a positive association with esophageal squamous cell carcinoma (HR for the highest tertile in the multivariable model: 1.76; 95% CI: 1.07, 2.90; *P*-trend = 0.01).

Heme iron intake was also positively associated with esophageal squamous cell carcinoma risk in men, with higher risk estimates in the multivariable-adjusted model (HR: 2.23; 95% CI: 1.05, 4.75; *P*-trend = 0.03) (**Table 3**). Risk estimates for the highest tertile of heme iron intake compared with the reference category also suggest an increased risk of gastric cardia adenocarcinoma, but the results were not significant and a trend was not seen. Furthermore, risk estimates among women do not indicate positive associations with any esophageal or gastric cancer subtype (Table 3). Inverse associations suggested by estimates for esophageal and gastric cardia adenocarcinoma are based on very few cases. A combined analysis of men and women showed no association with esophageal squamous cell carcinoma (HR for the highest tertile in the multivariable model: 1.27; 95% CI: 0.75, 2.14; *P*-trend = 0.38).

Cox regression models among men showed a positive association between nitrite and esophageal squamous cell carcinoma, but only continuous models were statistically significant (**Table 4**). Associations were not detected among women, and no association was seen for esophageal adenocarcinoma and gastric subtypes. A combined analysis of men and women showed no association with esophageal squamous cell carcinoma (HR for the highest tertile in the multivariable model: 1.33; 95% CI: 0.80, 2.23; *P*-trend = 0.28).

Analyses of nitrate intake and esophageal and gastric cancer subtypes are presented in **Table 5**. Significant associations were not seen for any esophageal or gastric cancer subtype. The HR for the highest tertile of nitrate intake for esophageal squamous cell carcinoma in models combining men and women was 1.04 (95% CI: 0.48, 2.25; *P*-trend = 0.90).

A combination of low vitamin C intake and high heme iron or nitrite intake compared with high vitamin C and low heme iron or nitrite intake showed positive associations with esophageal squamous cell carcinoma risk in men (*see* Supplementary Figures 1 and 2 under "Supplemental data" in the online issue). However, overall tests of interaction were not significant. In women, no associations were seen for esophageal squamous cell carcinoma or gastric noncardia adenocarcinoma across tertiles of vitamin C intake and heme iron or nitrite intake (data not shown). Tests of multiplicative effect modification of chlorophyll intake on the association between heme iron and the risk of esophageal and gastric cancer subtypes were not significant, and risk estimates across heme iron and chlorophyll categories did not indicate a trend consistent with our hypothesis (data not shown).

Among men who did not drink beer, the HR for esophageal squamous cell carcinoma comparing the highest tertile of *N*-nitrosodimethylamine intake with the lowest was 7.85 (95% CI: 1.10, 55.9). Exclusion of the first 2 y of follow-up, the use of age as time scale in the analysis, and alternative estimation of *N*-nitrosodimethylamine intake based on exclusion of noncheese dairy products did not substantially change our results (data not shown).

DISCUSSION

Data from this large prospective cohort show a positive association between esophageal squamous cell carcinoma risk and preformed dietary *N*-nitrosodimethylamine intake as well as nitrite and heme iron intake, which are proxies for endogenous nitrosation. There was also an indication of a positive association of *N*-nitrosodimethylamine with gastric noncardia adenocarcinoma. All associations were seen in men. In women, a positive association

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TABLE 1
Characteristics of cases and subcohort members in the Netherlands Cohort Study on diet and cancer, 1986–2002[1]

| | Men | | | | | Women | | | | |
| | Subcohort (n = 1947) | Cases | | | | Subcohort (n = 2085) | Cases | | | |
Characteristics		ESCC (n = 60)	EAC (n = 117)	GCA (n = 139)	GNCA (n = 330)		ESCC (n = 50)	EAC (n = 34)	GCA (n = 27)	GNCA (n = 167)
Age (y)	61.2 ± 4.2[2]	62.4 ± 4.1	61.4 ± 4.1	61.4 ± 4.1	62.4 ± 4.0	61.4 ± 4.2	62.6 ± 4.3	62.7 ± 4.2	62.6 ± 4.2	62.6 ± 4.3
BMI (kg/m^2)	24.9 ± 2.6	24.4 ± 3.5	26.1 ± 2.9	25.7 ± 2.7	24.7 ± 2.7	25.1 ± 3.5	24.1 ± 3.7	27.6 ± 4.6	26.1 ± 4.3	25.5 ± 4.1
Cigarette smoking (%)										
Never smoker	14	10	9	11	9	60	34	65	52	54
Former smoker	52	37	58	53	50	20	22	12	26	22
Current smoker	34	53	33	37	42	20	44	24	22	24
Level of education (%)										
Primary school	23	32	23	26	33	33	32	35	41	47
Lower vocational	20	20	27	24	24	23	24	26	30	24
High school	36	32	34	30	31	35	38	29	11	23
Higher vocational/university	20	17	16	19	12	9	6	9	19	5
Nonoccupational physical activity (%)										
<30 min/d	18	27	20	18	17	24	32	38	19	31
30 to <60 min/d	31	33	30	27	30	32	32	18	41	30
60 to <90 min/d	19	12	19	21	19	23	18	21	22	18
≥90 min/d	32	28	32	35	34	22	18	24	19	22
Family history of esophageal or gastric cancer (%)	8	8	12	9	12	7	10	12	7	15
Use of nonsteroidal antiinflammatory drugs (%)	6	3	7	4	4	8	12	9	11	7
Use of lower esophageal sphincter–relaxing medication (%)	16	17	26	16	17	14	14	3	15	15
Daily intake in subcohort										
Energy (kJ/d)	9085 ± 2129	8648 ± 2060	8977 ± 2062	8830 ± 2167	9168 ± 2111	7040 ± 1644	7458 ± 1874	7096 ± 1832	7482 ± 1437	6990 ± 1519
Alcohol (g/d)	9.6 (20.9)[3]	24.6 (33.0)	9.3 (22.9)	10.6 (21.2)	10.0 (21.5)	1.6 (7.8)	6.7 (25.5)	3.3 (13.9)	0.5 (4.1)	1.3 (7.4)
Beer (% users)	57	66	54	54	56	10	17	13	8	8
Fruit (g/d)	136 (134)	89 (101)	122 (135)	120 (130)	129 (128)	178 (145)	155 (176)	201 (136)	218 (148)	171 (150)
Vegetables (g/d)	188 (100)	161 (104)	185 (107)	187 (113)	177 (97)	190 (99)	223 (93)	178 (72)	222 (103)	192 (121)
Nitrate (mg/d)[4]	108.3 ± 45.0	95.6 ± 39.7	108.7 ± 49.7	104.3 ± 44.1	102.9 ± 38.7	105.9 ± 43.6	109.1 ± 45.0	93.7 ± 40.4	117.5 ± 46.6	103.9 ± 45.5
Nitrite (mg/d)[5]	0.12 (0.16)	0.15 (0.21)	0.11 (0.16)	0.13 (0.23)	0.13 (0.17)	0.08 (0.12)	0.07 (0.11)	0.07 (0.11)	0.08 (0.15)	0.08 (0.11)
NDMA (μg/d)	0.084 (0.121)	0.104 (0.340)	0.080 (0.100)	0.092 (0.110)	0.093 (0.166)	0.044 (0.032)	0.052 (0.046)	0.043 (0.035)	0.045 (0.038)	0.043 (0.028)
Heme iron (mg/d)	1.16 ± 0.51	1.28 ± 0.66	1.13 ± 0.49	1.21 ± 0.51	1.19 ± 0.49	0.97 ± 0.48	1.05 ± 0.57	0.91 ± 0.54	0.93 ± 0.57	0.96 ± 0.46
Chlorophyll (mg/d)	53.3 ± 30.0	48.0 ± 27.7	53.9 ± 32.0	52.4 ± 32.7	50.9 ± 27.5	52.9 ± 29.8	52.9 ± 32.9	45.8 ± 27.1	59.7 ± 31.3	50.5 ± 27.3
Vitamin C (mg/d)	99.3 ± 43.3	82.0 ± 42.8	96.6 ± 46.9	87.8 ± 34.9	94.4 ± 42.3	109.0 ± 44.4	111.3 ± 43.3	107.4 ± 47.2	114.6 ± 52.7	109.4 ± 49.7
ENOC (μg/d)	102.0 ± 25.2	107.3 ± 27.6	100.8 ± 24.2	104.4 ± 25.1	103.6 ± 24.3	92.9 ± 23.2	96.5 ± 28.1	89.7 ± 26.5	90.9 ± 28.2	92.3 ± 22.8

[1] Percentages do not add up to 100 because of rounding. EAC, esophageal adenocarcinoma; ENOC, index of endogenous N-nitrosation; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric noncardia adenocarcinoma; NDMA, N-nitrosodimethylamine.
[2] Mean ± SD (all such values).
[3] Median; IQR in parentheses (all such values).
[4] Total nitrate intake from food and water.
[5] Nitrite intake from processed meat.

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was only seen between *N*-nitrosodimethylamine and esophageal squamous cell carcinoma, possibly because of lower ranges of exposure, especially for *N*-nitrosodimethylamine, and smaller sample sizes.

Few epidemiologic studies have investigated esophageal cancer risk in relation to NOC intake and nitrosation, and their results are inconsistent. One case-control study from the United States found a nonsignificant positive association between esophageal cancer risk and nitrite intake (OR for the highest category: 1.58; 95% CI: 0.73, 3.44) as well as *N*-nitrosodimethylamine intake (OR for the highest category: 1.86; 95% CI: 0.87, 3.95) (36). A cohort study in a United Kingdom population with 55 esophageal cancer cases showed no association with intake of *N*-nitrosodimethylamine, intake of nitrite, and endogenous nitrosation (10), although subtypes were not distinguished in this study. A cohort study (37) and a case-control study (38) from the United States also suggested no association with nitrite intake. In a cohort study that investigated heme iron intake in relation to esophageal cancer, a suggestive positive association was found with adenocarcinoma but not with squamous cell carcinoma of the esophagus (37). The same study found a positive association between squamous cell carcinoma and red meat intake (37), which we also observed in the NLCS (39). Another cohort study of women in Iowa found a positive association between heme iron intake and upper digestive tract cancer (esophageal and stomach cancers) (40), and 2 case-control studies showed a positive association with esophageal adenocarcinoma (41, 42).

Several studies have investigated the relation between gastric cancer risk and NOC intake and nitrosation (7). Four case-control studies (43–46) showed a positive association with *N*-nitrosodimethylamine intake, whereas one study (47) found no association. One (9) of 2 Scandinavian cohort studies found a positive association between *N*-nitrosodimethylamine intake and gastric cancer risk (9, 48), and no association with either cardia or noncardia adenocarcinoma was found in a large European cohort from 10 countries (8). Most of the case-control studies (6, 49), but not all (43, 46), showed a consistent positive association between nitrite intake and gastric cancer risk, whereas 3 cohort studies did not support an association (10, 37, 48). After 6.3 y of follow-up, we observed a clear association with nitrite intake in the NLCS (23). Few studies had distinguished between cardia and noncardia gastric cancers (8, 37, 38). A case-control study showed an association between nitrite intake and noncardia adenocarcinoma, but not with cardia adenocarcinoma (38), whereas a cohort study found no association with either gastric subtype (37). Endogenous nitrosation, based on total iron intake from meat, was found to be associated with gastric noncardia but not with gastric cardia cancer in a European cohort study (8); however, heme iron intake was not associated with gastric cancer subtypes in a large US cohort study (37).
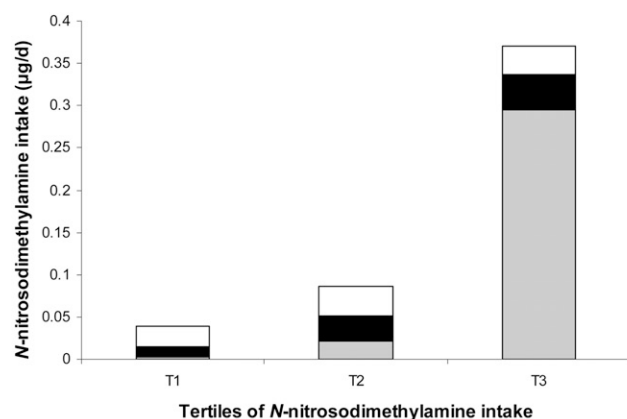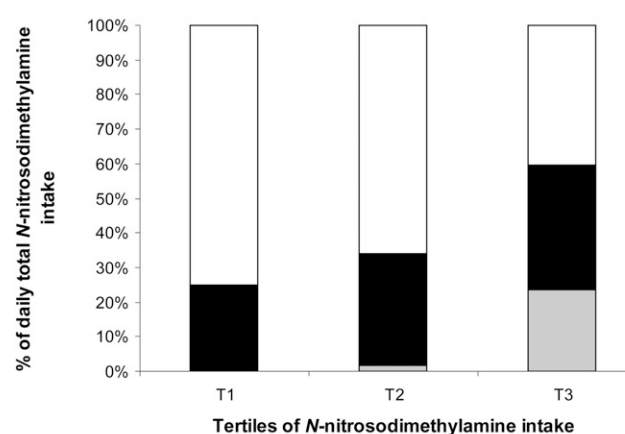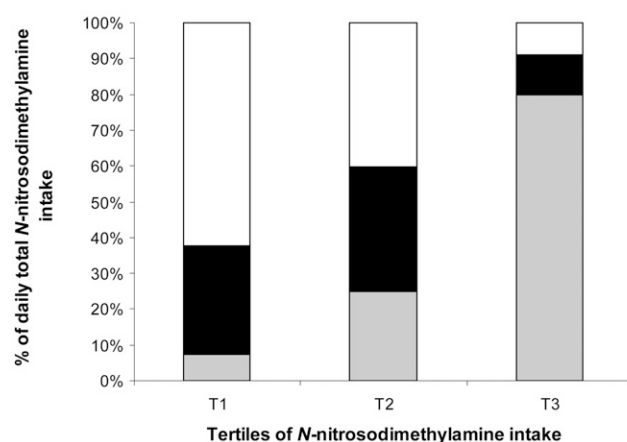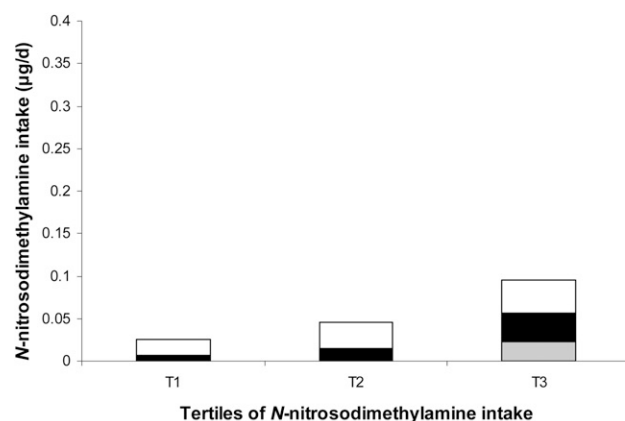
FIGURE 2. Absolute and relative contribution of beer (gray bars), processed meat (black bars), and other foods and beverages (white bars) to the total *N*-nitrosodimethylamine intake by sex in the subcohort of the Netherlands Cohort Study, 1986–2002 (*n* = 1947 and 2085 for men and women, respectively). T, tertile.

TABLE 2
HRs and 95% CIs of esophageal and gastric cancer subtypes in men and women by N-nitrosodimethylamine intake in the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	Men					Women				
	Tertile			P-trend	Continuous (0.1-μg/d increment)	Tertile			P-trend	Continuous (0.1-μg/d increment)
	1	2	3			1	2	3		
Median intake (μg/d)	0.04	0.08	0.25			0.03	0.04	0.07		
Person-years in subcohort	8697	9042	8971			10,308	10,507	10,248		
Esophageal squamous cell carcinoma										
No. of cases	16	15	29			15	14	21		
HR[1] (95% CI)	1	0.99 (0.48, 2.04)	2.09 (1.08, 4.03)	<0.01	1.14 (1.08, 1.21)	1	0.93 (0.44, 1.94)	1.52 (0.77, 3.01)	0.19	1.41 (1.15, 1.74)
HR[2] (95% CI)	1	1.21 (0.54, 2.74)	2.43 (1.13, 5.23)	0.01	1.15 (1.05, 1.25)	1	0.92 (0.43, 1.97)	1.21 (0.56, 2.62)	0.57	1.34 (1.04, 1.71)
Esophageal adenocarcinoma										
No. of cases	40	39	38			13	10	11		
HR[1] (95% CI)	1	0.96 (0.61, 1.52)	0.97 (0.60, 1.55)	0.92	1.02 (0.95, 1.10)	1	0.77 (0.33, 1.77)	0.93 (0.41, 2.13)	0.91	0.96 (0.59, 1.57)
HR[2,3] (95% CI)	1	0.93 (0.57, 1.50)	0.87 (0.52, 1.45)	0.63	0.98 (0.91, 1.06)	1	0.79 (0.32, 1.91)	0.92 (0.40, 2.14)	0.90	0.92 (0.55, 1.54)
Gastric cardia adenocarcinoma										
No. of cases	47	47	45			8	9	10		
HR[1] (95% CI)	1	0.98 (0.64, 1.50)	0.97 (0.63, 1.49)	0.90	1.02 (0.96, 1.09)	1	1.12 (0.43, 2.89)	1.36 (0.54, 3.42)	0.51	0.90 (0.56, 1.44)
HR[2] (95% CI)	1	1.00 (0.64, 1.56)	0.94 (0.59, 1.49)	0.75	1.01 (0.94, 1.08)	1	0.97 (0.34, 2.78)	1.02 (0.33, 3.14)	0.96	0.65 (0.23, 1.84)
Gastric noncardia adenocarcinoma										
No. of cases	100	105	125			53	70	44		
HR[1] (95% CI)	1	1.09 (0.81, 1.47)	1.41 (1.05, 1.90)	0.02	1.07 (1.03, 1.11)	1	1.32 (0.91, 1.91)	0.90 (0.59, 1.37)	0.47	0.77 (0.54, 1.10)
HR[2] (95% CI)	1	1.09 (0.79, 1.50)	1.31 (0.95, 1.81)	0.09	1.06 (1.01, 1.10)	1	1.37 (0.92, 2.02)	0.90 (0.58, 1.42)	0.49	0.74 (0.51, 1.06)

[1] Adjusted for age (y); calculated by using a Cox proportional hazards model.
[2] Adjusted for age (y), smoking status (current or noncurrent smoker), years of cigarette smoking, number of cigarettes smoked per day, total energy intake (kJ/d), BMI (in kg/m^2; <20, 20–24.9, 25–29.9, or ≥30), alcoholic beverages not including beer (g/d), vegetable intake (g/d), fruit intake (g/d), level of education (4 categories), and nonoccupational physical activity (4 categories). Calculated by using a Cox proportional hazards model.
[3] Models additionally adjusted for use of lower esophageal sphincter–relaxing medications.

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TABLE 3
HRs and 95% CIs of esophageal and gastric cancer subtypes in men and women by heme iron intake in the Netherlands Cohort Study on Diet and Cancer, 1986–2002[1]

	Men					Women				
	Tertile			P-trend	Continuous (1-mg/d increment)	Tertile			P-trend	Continuous (1-mg/d increment)
	1	2	3			1	2	3		
Median intake (mg/d)	0.71	1.09	1.62			0.56	0.92	1.32		
Median ENOC[2] (μg/d)	80	98.6	124.8			72.8	90.5	110.2		
Person-years in subcohort	8688	8933	8827			9894	9900	9983		
Esophageal squamous cell carcinoma										
No. of cases	14	21	24			17	14	17		
HR[3] (95% CI)	1	1.47 (0.74, 2.91)	1.75 (0.90, 3.41)	0.10	1.55 (0.93, 2.58)	1	0.83 (0.40, 1.70)	1.00 (0.50, 1.98)	0.98	1.24 (0.73, 2.11)
HR[4] (95% CI)	1	1.74 (0.81, 3.74)	2.23 (1.05, 4.75)	0.03	1.83 (0.98, 3.39)	1	0.75 (0.34, 1.64)	0.71 (0.34, 1.51)	0.40	1.02 (0.52, 2.02)
Esophageal adenocarcinoma										
No. of cases	37	42	35			13	11	7		
HR[3] (95% CI)	1	1.11 (0.70, 1.74)	0.94 (0.58, 1.51)	0.74	0.92 (0.64, 1.33)	1	0.85 (0.38, 1.92)	0.54 (0.21, 1.36)	0.18	0.57 (0.21, 1.59)
HR[4,5] (95% CI)	1	1.04 (0.64, 1.68)	0.81 (0.47, 1.40)	0.41	0.79 (0.51, 1.23)	1	0.71 (0.28, 1.76)	0.39 (0.15, 1.01)	0.05	0.40 (0.12, 1.34)
Gastric cardia adenocarcinoma										
No. of cases	36	52	51			14	4	6		
HR[3] (95% CI)	1	1.41 (0.91, 2.18)	1.40 (0.90, 2.18)	0.16	1.18 (0.87, 1.61)	1	0.29 (0.09, 0.88)	0.43 (0.16, 1.12)	0.10	0.52 (0.14, 1.90)
HR[4] (95% CI)	1	1.46 (0.93, 2.31)	1.52 (0.96, 2.42)	0.09	1.25 (0.89, 1.77)	1	0.26 (0.08, 0.81)	0.32 (0.11, 0.91)	0.05	0.38 (0.09, 1.61)
Gastric noncardia adenocarcinoma										
No. of cases	93	120	116			55	50	55		
HR[3] (95% CI)	1	1.26 (0.94, 1.69)	1.27 (0.95, 1.71)	0.14	1.14 (0.92, 1.41)	1	0.92 (0.61, 1.37)	1.00 (0.68, 1.48)	0.98	0.96 (0.70, 1.32)
HR[4] (95% CI)	1	1.25 (0.91, 1.71)	1.25 (0.90, 1.73)	0.21	1.12 (0.87, 1.44)	1	0.93 (0.62, 1.40)	0.96 (0.63, 1.44)	0.84	0.90 (0.64, 1.26)

[1] An additional 129 individuals were excluded from the analysis because of missing alcohol intake values.
[2] ENOC, index of endogenous N-nitroso compound formation.
[3] Adjusted for age (y); calculated by using a Cox proportional hazards model.
[4] Adjusted for age (y), smoking status (current or noncurrent smoker), years of cigarette smoking, number of cigarettes smoked per day, total energy intake (kJ/d), BMI (in kg/m^2; <20, 20–24.9, 25–29.9, or ≥30), alcohol intake (g/d), vegetable intake (g/d), fruit intake (g/d), level of education (4 categories), and nonoccupational physical activity (4 categories). Calculated by using a Cox proportional hazards model.
[5] Models were additionally adjusted for use of lower esophageal sphincter–relaxing medications.

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TABLE 4
HRs and 95% CIs of esophageal and gastric cancer subtypes in men and women by nitrite intake in the Netherlands Cohort Study on Diet and Cancer, 1986–2002[1]

| | Men | | | | | Women | | | | |
| | Tertile | | | | Continuous | Tertile | | | | Continuous |
	1	2	3	*P*-trend	(0.1-mg/d increment)	1	2	3	*P*-trend	(0.1-mg/d increment)
Median intake (mg/d)[2]	0.03	0.12	0.28			0.02	0.08	0.2		
Person-years in subcohort	8665	8892	8890			10,009	10,016	9752		
Esophageal squamous cell carcinoma										
No. of cases	17	19	23			16	18	14		
HR[3] (95% CI)	1	1.18 (0.61, 2.30)	1.49 (0.78, 2.87)	0.22	1.09 (0.98, 1.22)	1	1.17 (0.59, 2.32)	0.96 (0.46, 2.00)	0.82	0.89 (0.69, 1.16)
HR[4] (95% CI)	1	1.27 (0.62, 2.62)	1.92 (0.94, 3.89)	0.06	1.19 (1.05, 1.36)	1	0.99 (0.48, 2.03)	0.85 (0.39, 1.88)	0.67	0.83 (0.61, 1.12)
Esophageal adenocarcinoma										
No. of cases	42	38	34			12	12	7		
HR[3] (95% CI)	1	0.90 (0.57, 1.43)	0.81 (0.50, 1.31)	0.41	0.94 (0.83, 1.06)	1	1.05 (0.47, 2.36)	0.64 (0.25, 1.64)	0.31	0.95 (0.65, 1.39)
HR[4,5] (95% CI)	1	0.86 (0.53, 1.39)	0.74 (0.43, 1.28)	0.30	0.91 (0.78, 1.06)	1	0.92 (0.39, 2.16)	0.61 (0.25, 1.53)	0.27	0.94 (0.63, 1.39)
Gastric cardia adenocarcinoma										
No. of cases	47	39	53			9	9	6		
HR[3] (95% CI)	1	0.83 (0.53, 1.29)	1.14 (0.75, 1.72)	0.42	1.06 (0.97, 1.15)	1	1.05 (0.41, 2.67)	0.73 (0.26, 2.07)	0.52	0.93 (0.61, 1.42)
HR[4] (95% CI)	1	0.80 (0.51, 1.27)	1.18 (0.75, 1.86)	0.34	1.07 (0.97, 1.19)	1	0.97 (0.36, 2.58)	0.62 (0.20, 1.90)	0.37	0.85 (0.53, 1.37)
Gastric noncardia adenocarcinoma										
No. of cases	98	109	122			56	50	54		
HR[3] (95% CI)	1	1.17 (0.87, 1.58)	1.36 (1.01, 1.82)	0.04	1.06 (1.00, 1.13)	1	0.94 (0.63, 1.39)	1.06 (0.71, 1.57)	0.71	0.97 (0.84, 1.11)
HR[4] (95% CI)	1	1.10 (0.80, 1.50)	1.23 (0.89, 1.70)	0.20	1.04 (0.97, 1.12)	1	0.94 (0.62, 1.41)	1.08 (0.71, 1.63)	0.65	0.96 (0.83, 1.12)

[1] An additional 129 individuals were excluded from the analysis because of missing alcohol intake values.
[2] Nitrite intake from processed meat.
[3] Adjusted for age (y); calculated by using a Cox proportional hazards model.
[4] Adjusted for age (y), smoking status (current or noncurrent smoker), years of cigarette smoking, number of cigarettes smoked per day, total energy intake (kJ/d), BMI (in kg/m^2; <20, 20–24.9, 25–29.9, or ≥30), alcoholic intake (g/d), vegetable intake (g/d), fruit intake (g/d), level of education (4 categories), and nonoccupational physical activity (4 categories). Calculated by using a Cox proportional hazards model.
[5] Models were additionally adjusted for use of lower esophageal sphincter–relaxing medications.

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TABLE 5
HRs and 95% CIs of esophageal and gastric cancer subtypes in men and women by nitrate intake in the Netherlands Cohort Study on Diet and Cancer, 1986–2002[1]

	Men					Women				
	Tertile				Continuous	Tertile				Continuous
	1	2	3	P-trend	(10-mg/d increment)	1	2	3	P-trend	(10-mg/d increment)
Median intake (mg/d)	68.1	100.8	146.2			66.4	98.5	142.7		
Person-years in subcohort	8383	9015	9050			9607	10,175	9996		
Esophageal squamous cell carcinoma										
No. of cases	24	21	14			15	18	15		
HR[2] (95% CI)	1	0.82 (0.45, 1.48)	0.54 (0.28, 1.05)	0.07	0.92 (0.85, 1.00)	1	1.17 (0.58, 2.35)	1.02 (0.49, 2.14)	0.99	1.02 (0.96, 1.08)
HR[3] (95% CI)	1	1.50 (0.72, 3.12)	1.51 (0.49, 4.62)	0.43	1.05 (0.92, 1.21)	1	1.17 (0.55, 2.47)	0.75 (0.23, 2.40)	0.60	1.00 (0.88, 1.14)
Esophageal adenocarcinoma										
No. of cases	39	36	39			14	13	4		
HR[2] (95% CI)	1	0.86 (0.54, 1.37)	0.92 (0.58, 1.46)	0.77	1.00 (0.95, 1.05)	1	0.89 (0.42, 1.92)	0.29 (0.09, 0.89)	0.02	0.89 (0.80, 1.00)
HR[3,4] (95% CI)	1	0.95 (0.58, 1.55)	0.94 (0.50, 1.77)	0.86	1.00 (0.92, 1.09)	1	0.90 (0.35, 2.28)	0.26 (0.05, 1.37)	0.11	0.86 (0.71, 1.04)
Gastric cardia adenocarcinoma										
No. of cases	49	47	43			7	7	10		
HR[2] (95% CI)	1	0.89 (0.59, 1.35)	0.81 (0.53, 1.24)	0.33	0.98 (0.93, 1.02)	1	0.97 (0.34, 2.81)	1.46 (0.54, 3.93)	0.42	1.04 (0.96, 1.12)
HR[3] (95% CI)	1	1.06 (0.68, 1.65)	1.01 (0.57, 1.77)	0.99	1.00 (0.92, 1.08)	1	1.01 (0.30, 3.42)	1.61 (0.32, 8.06)	0.54	1.07 (0.90, 1.28)
Gastric noncardia adenocarcinoma										
No. of cases	111	125	93			59	46	55		
HR[2] (95% CI)	1	1.05 (0.79, 1.39)	0.77 (0.57, 1.04)	0.07	0.97 (0.94, 1.00)	1	0.76 (0.51, 1.13)	0.95 (0.64, 1.40)	0.89	1.00 (0.96, 1.04)
HR[3] (95% CI)	1	1.23 (0.90, 1.68)	1.05 (0.70, 1.59)	0.80	1.00 (0.95, 1.05)	1	0.73 (0.47, 1.11)	0.78 (0.44, 1.39)	0.43	0.96 (0.89, 1.04)

[1] An additional 129 individuals were excluded from the analysis because of missing alcohol intake values.
[2] Adjusted for age (y); calculated by using a Cox proportional hazards model.
[3] Adjusted for age (y), smoking status (current or noncurrent smoker), years of cigarette smoking, number of cigarettes smoked per day, total energy intake (kJ/d), BMI (in kg/m^2; <20, 20–24.9, 25–29.9, or ≥30), alcoholic intake (g/d), vegetable intake (g/d), fruit intake (g/d), level of education (4 categories), and nonoccupational physical activity (4 categories). Calculated by using a Cox proportional hazards model.
[4] Models were additionally adjusted for use of lower esophageal sphincter–relaxing medications.

As in most previous studies, we used estimates of N-nitrosodimethylamine, the most common volatile nitrosamine in foods (1), as an indicator of dietary exposure to preformed NOCs. The distribution of N-nitrosodimethylamine intake in our study was largely similar to that of other studies (8, 9). It is difficult, however, to estimate the N-nitrosodimethylamine intake of individuals based on questionnaire and published data on N-nitrosodimethylamine contents of food. The nitrosamine contents of food vary across countries (20) and over time, because of changes in methods of food processing (50), and available data are limited for some food items. Nonetheless, our analysis with different N-nitrosodimethylamine estimates yielded similar results.

Beer and processed meat were the most important source of N-nitrosodimethylamine in the NLCS (Figure 2), which is in line with other studies (9, 36, 48). Before the 1980s, beer contained considerable amounts of N-nitrosodimethylamine in the Netherlands and in other countries (12), although the contents have decreased substantially since then owing to measures taken by brewers to reduce N-nitrosodimethylamine formation in malt (51). Given the long latency periods, we might still pick up effects of beer consumption in the past. The low nitrite content of beer (19), in contrast with its high N-nitrosodimethylamine content, might partially explain the differences in associations that we found with N-nitrosodimethylamine and nitrite intake and might indicate different underlying mechanisms leading to cancer.

Alcohol consumption is associated with the risk of esophageal squamous cell carcinoma (52). Because there was a strong correlation between alcohol in beer and N-nitrosodimethylamine intake in our study, we evaluated the possibility that the association shown in this study was due to alcohol in beer rather than to N-nitrosodimethylamine intake by restricting the analysis to non–beer drinkers. These analyses also showed a strong positive association between N-nitrosodimethylamine and esophageal squamous cell carcinoma among men, which supports the role of N-nitrosodimethylamine in the association.

We assessed the possible effect of endogenous nitrosation on esophageal and gastric cancer risk by using nitrite and heme iron intakes, which have been shown to influence NOC formation (4). An ENOC formation using iron intake has been developed by Jakszyn et al (8) and was used in several studies. We used a similar estimation of ENOC using heme iron instead of iron intake from meat. The correlation between heme iron and ATNC levels was very high ($r = 0.97$), which suggests that this approach is useful in estimating endogenous NOC formation in the distal gastrointestinal tract.

The intake of vitamin C, which is known to inhibit nitrosation, can reduce the endogenous formation of NOCs (53). Several epidemiologic studies found an effect modification of nitrite, preformed nitrosamine, or endogenous nitrosation by vitamin C intake on gastric and esophageal cancer (8, 36, 38, 44, 54). Our results are consistent with effect modification only for esophageal squamous cell carcinoma risk. We found no effect modification between heme iron and chlorophyll intake. Chlorophyll is structurally similar to heme and can reduce carcinogenesis in animal models (55). Experiments in rats have shown that chlorophyll inhibits the cytotoxic and hyperproliferative effects of dietary heme in the colon (5).

The prospective design and the nearly complete follow-up is a key strength of this study for reducing the potential for bias. The effect of preclinical disease on exposure was not likely to introduce substantial error, which is supported by similar findings when early follow-up was excluded from the analysis. The collection and analysis of detailed data on potential confounders is another strength. However, food-frequency questionnaires are prone to the misclassification of dietary intake; hence, we could have missed relevant associations. We performed a separate analysis of esophageal and gastric cancer subtypes, and our results suggest different risks of NOCs across subtypes, but we could not perform an analysis by histology of gastric adenocarcinomas. The lack of information on cooking methods and *Helicobacter pylori* infection status were also limitations of our study. We estimate that about half of our study population could have been infected (56), but it is unlikely that risk estimates for esophageal squamous cell carcinoma have been attenuated.

We concluded from the results of this large Dutch cohort study that preformed NOC, heme iron intake, and nitrite intake may increase the risk of esophageal squamous cell carcinoma in men, but no apparent associations were seen for esophageal adenocarcinoma. Our results also support a positive association between preformed N-nitrosodimethylamine and gastric noncardia adenocarcinoma. Overall, the results in women do not support a positive association with esophageal or gastric cancer subtypes.

REFERENCES

1. Tricker AR. N-nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev 1997;6:226–68.
2. Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. Cancer Lett 1995;93:17–48.
3. International Agency for Research on Cancer. Overall evaluation of carcinogenicity to humans. IARC monographs. Vol. 1-82. Lyon, France: IARC.
4. Cross AJ, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 2003;63:2358–60.
5. de Vogel J, Jonker-Termont DS, van Lieshout EM, Katan MB, van der Meer R. Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis 2005;26:387–93.
6. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Ingested nitrate and nitrite, and cyanobacterial peptide toxins. Vol 94. Lyon, France: IARC, 2010.
7. Jakszyn P, Gonzalez CA. Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. World J Gastroenterol 2006;12:4296–303.
8. Jakszyn P, Bingham S, Pera G, Agudo A, Luben R, Welch A, Boeing H, Del Giudice G, Palli D, Saieva C, et al. Endogenous versus exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. Carcinogenesis 2006;27:1497–501.

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9. Larsson SC, Bergkvist L, Wolk A. Processed meat consumption, dietary nitrosamines and stomach cancer risk in a cohort of Swedish women. Int J Cancer 2006;119:915–9.

10. Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. Am J Clin Nutr 2011;93:1053–61.

11. Stephany RW, Schuller PL. Daily dietary intakes of nitrate, nitrite and volative N-nitrosamines in the Netherlands using the duplicate portion sampling technique. Oncology 1980;37:203–10.

12. Tricker AR, Preussmann R. Volatile and nonvolatile nitrosamines in beer. J Cancer Res Clin Oncol 1991;117:130–2.

13. van den Brandt PA, Goldbohm RA, van 't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in the Netherlands. J Clin Epidemiol 1990;43:285–95.

14. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika 1986;73:1–11.

15. Van den Brandt PA, Schouten LJ, Goldbohm RA, Dorant E, Hunen PM. Development of a record linkage protocol for use in the Dutch Cancer Registry for Epidemiological Research. Int J Epidemiol 1990;19:553–8.

16. Schouten LJ, Hoppener P, van den Brandt PA, Knottnerus JA, Jager JJ. Completeness of cancer registration in Limburg, the Netherlands. Int J Epidemiol 1993;22:369–76.

17. World Health Organization. International classification of diseases for oncology. 3rd ed. Geneva, Switzerland: World Health Organization, 2000.

18. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F, Hermus RJ. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. Eur J Clin Nutr 1994;48:253–65.

19. Cornee J, Lairon D, Velema J, Guyader M, Berthezene P. An estimate of nitrate, nitrite, and N-nitrosodimethylamine concentrations in French food-products or food groups. Sci Aliments 1992;12:155–97.

20. Jakszyn P, Agudo A, Ibanez R, Garcia-Closas R, Pera G, Amiano P, Gonzalez CA. Development of a food database of nitrosamines, heterocyclic amines, and polycyclic aromatic hydrocarbons. J Nutr 2004;134:2011–4.

21. Osterdahl BG. Volatile nitrosamines in foods on the Swedish market and estimation of their daily intake. Food Addit Contam 1988;5:587–95.

22. Tricker AR, Pfundstein B, Theobald E, Preussmann R, Spiegelhalder B. Mean daily intake of volatile N-nitrosamines from foods and beverages in West Germany in 1989-1990. Food Chem Toxicol 1991;29:729–32.

23. van Loon AJ, Botterweck AA, Goldbohm RA, Brants HA, van Klaveren JD, van den Brandt PA. Intake of nitrate and nitrite and the risk of gastric cancer: a prospective cohort study. Br J Cancer 1998;78:129–35.

24. Driessen JJM. Nitraat en nitrietgehalte van een aantal rauwe en toebereide groenten. (Nitrate and nitrite content of a number of raw and cooked vegetables.) Wageningen, Netherlands: State Institute for Quality Control of Agricultural Products (RIKILT), 1989 (in Dutch).

25. van de Worp HHM. Onderzoek naar de gehalte aan nitraat en nitriet in een aantal wintergroenten. (Examination of the nitrate and nitrite content of various winter vegetables.) Wageningen, Netherlands: State Institute for Quality Control of Agricultural Products (RIKILT), 1987 (in Dutch).

26. Ellen G, Egmond E, Sahertian ET. Volatile N-nitrosamines and nitrite in cured meats. RIVM Report no. 648305001, 1984 (in Dutch). Bilthoven, Netherlands: The National Institute for Public Health and the Environment.

27. NEVO-tabel: Nederlands voedingsstoffenbestand 1986-1987. (Nevo-table: Dutch food composition table 1986-1987.) The Hague, Netherlands: Voorlichtingsbureau voor de voeding, 1986 (in Dutch).

28. Balder HF, Vogel J, Jansen MC, Weijenberg MP, van den Brandt PA, Westenbrink S, van der Meer R, Goldbohm RA. Heme and chlorophyll intake and risk of colorectal cancer in the Netherlands cohort study. Cancer Epidemiol Biomarkers Prev 2006;15:717–25.

29. Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. J Nutr 2002;132:3522S–5S.

30. Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill IK. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996;17:515–23.

31. Hughes R, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis 2001;22:199–202.

32. Silvester KR, Bingham SA, Pollock JR, Cummings JH, O'Neill IK. Effect of meat and resistant starch on fecal excretion of apparent N-nitroso compounds and ammonia from the human large bowel. Nutr Cancer 1997;29:13–23.

33. Lin DY, Wei LJ. The robust inference for the Cox proportional hazards model. J Am Stat Assoc 1989;84:1074–8.

34. Schoenfeld D. Partial residuals for the proportional hazards regression model. Biometrika 1982;69:239–41.

35. Cleves MA, Gould WW, Gutierrez RG, Marchenko YU. An introduction to survival analysis using Stata. 2nd ed. College Station, TX: Stata Press, 2008.

36. Rogers MA, Vaughan TL, Davis S, Thomas DB. Consumption of nitrate, nitrite, and nitrosodimethylamine and the risk of upper aerodigestive tract cancer. Cancer Epidemiol Biomarkers Prev 1995;4:29–36.

37. Cross AJ, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, Sinha R, Abnet CC. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. Am J Gastroenterol 2011;106:432–42.

38. Mayne ST, Risch HA, Dubrow R, Chow WH, Gammon MD, Vaughan TL, Farrow DC, Schoenberg JB, Stanford JL, Ahsan H, et al. Nutrient intake and risk of subtypes of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 2001;10:1055–62.

39. Keszei AP, Schouten LJ, Goldbohm RA, van den Brandt PA. Red and processed meat consumption and the risk of esophageal and gastric cancer subtypes in The Netherlands Cohort Study. Ann Oncol 2012;23:2319–26.

40. Lee DH, Anderson KE, Folsom AR, Jacobs DR Jr. Heme iron, zinc and upper digestive tract cancer: the Iowa Women's Health Study. Int J Cancer 2005;117:643–7.

41. O'Doherty MG, Abnet CC, Murray LJ, Woodside JV, Anderson LA, Brockman JD, Cantwell MM. Iron intake and markers of iron status and risk of Barrett's esophagus and esophageal adenocarcinoma. Cancer Causes Control 2010;21:2269–79.

42. Ward MH, Cross AJ, Abnet CC, Sinha R, Markin RS, Weisenburger DD. Heme iron from meat and risk of adenocarcinoma of the esophagus and stomach. Eur J Cancer Prev 2012;21:134–8.

43. De Stefani E, Boffetta P, Mendilaharsu M, Carzoglio J, Deneo-Pellegrini H. Dietary nitrosamines, heterocyclic amines, and risk of gastric cancer: a case-control study in Uruguay. Nutr Cancer 1998;30:158–62.

44. González CA, Riboli E, Badosa J, Batiste E, Cardona T, Pita S, Sanz JM, Torrent M, Agudo A. Nutritional factors and gastric cancer in Spain. Am J Epidemiol 1994;139:466–73.

45. La Vecchia C, D'Avanzo B, Airoldi L, Braga C, Decarli A. Nitrosamine intake and gastric cancer risk. Eur J Cancer Prev 1995;4:469–74.

46. Pobel D, Riboli E, Cornee J, Hemon B, Guyader M. Nitrosamine, nitrate and nitrite in relation to gastric cancer: a case-control study in Marseille, France. Eur J Epidemiol 1995;11:67–73.

47. Risch HA, Jain M, Choi NW, Fodor JG, Pfeiffer CJ, Howe GR, Harrison LW, Craib KJ, Miller AB. Dietary factors and the incidence of cancer of the stomach. Am J Epidemiol 1985;122:947–59.

48. Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int J Cancer 1999;80:852–6.

49. Hernández-Ramirez RU, Galvan-Portillo MV, Ward MH, Agudo A, Gonzalez CA, Onate-Ocana LF, Herrera-Goepfert R, Palma-Coca O, Lopez-Carrillo L. Dietary intake of polyphenols, nitrate and nitrite and gastric cancer risk in Mexico City. Int J Cancer 2009;125:1424–30.

50. Hotchkiss JH. Preformed N-nitroso compounds in foods and beverages. Cancer Surv 1989;8:295–321.

51. Ellen G, Egmond E, Van Loon JW, Sahertian ET, Tolsma K. Dietary intakes of some essential and non-essential trace elements, nitrate, nitrite and N-nitrosamines, by Dutch adults: estimated via a 24-hour duplicate portion study. Food Addit Contam 1990;7:207–21.

52. Steevens J, Schouten LJ, Goldbohm RA, van den Brandt PA. Alcohol consumption, cigarette smoking and risk of subtypes of oesophageal and gastric cancer: a prospective cohort study. Gut 2010;59:39–48.

53. Mirvish SS. Effects of vitamins C and E on N-nitroso compound formation, carcinogenesis, and cancer. Cancer 1986;58:1842–50.

54. Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Bonaguri C, Cipriani F, Cocco P, Giacosa A, et al. A case-control study of gastric cancer and diet in Italy: II. Association with nutrients. Int J Cancer 1990;45:896–901.

55. Simonich MT, McQuistan T, Jubert C, Pereira C, Hendricks JD, Schimerlik M, Zhu B, Dashwood RH, Williams DE, Bailey GS. Low-dose dietary chlorophyll inhibits multi-organ carcinogenesis in the rainbow trout. Food Chem Toxicol 2008;46:1014–24.

56. Taylor DN, Blaser MJ. The epidemiology of Helicobacter pylori infection. Epidemiol Rev 1991;13:42–59.